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# Direct injection of seawater for the analysis of nitroaromatic explosives and their degradation products by micellar electrokinetic chromatography

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ABSTRACT

Practical considerations for the injection and separation of nitroaromatic explosives in seawater sample matrices are discussed. The use of high surfactant concentrations and long electrokinetic injections allows for improved detection limits. Sensitivity was enhanced by two mechanisms, improved stacking at the detector-side of the sample plug and desorption of analyte from the capillary wall by surfactant-containing BGE from the inlet side of the sample plug. Calculated limits of detection (S/N = 3) for analytes prepared in pure seawater were 70–800 ppb with injection times varying from 5 to 100 s.



## 1. Introduction

Methods for improving the detection limits of nitroaromatic explosives and their degradation products in seawater continue to be an ongoing concern of the U.S. Navy. The presence of these materials in coastal waters may be indicative of underwater mines that are military, commercial and environmental threats to our coastal regions. Micellar electrokinetic chromatography (MEKC) has been demonstrated to be a useful analytical tool in the analysis of nitroaromatics. Our group and others have demonstrated that MEKC-based separations not only allow for the resolution of the analytes of interest, but also preconcentration of the sample via sweeping or high-salt stacking [1–7]. On-line preconcentration allows for longer injection volumes which improve both detection limits and resolving power due to analyte preconcentration at the interface between the sample zone and the micelles in the background electrolyte (BGE).

The underlying challenge associated with nitroaromatic explosives detection in seawater is the presence of the matrix, itself, which has a basic pH ~8, contains several organic materials, and bears a large number of charged ions at very high concentrations. The primary difficulty, from an electrophoresis standpoint, is the large difference in sample matrix conductivity relative to BGE conductivity that arises when a seawater sample is directly injected onto the capillary column either by hydrodynamic or electrokinetic means. Relatively high sample matrix conductivity can lead to broadening due to electrodispersion, joule heating, and disruption of the sample zone/BGE interface needed for sample preconcentration [8,9]. Our previous efforts to detect explosives directly in seawater focused on implementing high-salt stacking in MEKC as a means of on-column preconcentration [2]. While direct sampling from seawater was possible, the best calculated limit of detection was demonstrated when using a diluted matrix, i.e. a 25% seawater sample matrix. The detection limits for many of the nitroaromatics were ~200 ppb in 25% seawater using a 75 μm I.D. capillary, or ~800 ppb when accounting for the sample dilution [2]. It should be noted that some groups have demonstrated calculated LODs in the range of tens of parts per billion on microchip-based platforms [10,11] and as low as 7 ppb for TNT in flow-through systems that deliver analyte to a detection electrode without the benefit of separation (no surfactant is included in the background electrolyte) [12]; however, those methods were not tested with seawater sample matrices. In many cases the optimized separation buffer will not tolerate a high conductivity sample matrix. For example, Chen and co-workers calculated LODs ranging from 12 to 52 ppb on a microchip-based system using amperometric detection using 15 mM sodium tetraborate, 15 mM SDS as the separation media [11]. Our efforts in understanding the role of micelle stacking have demonstrated that SDS containing background electrolytes are typically not useful in the analysis of high-salt matrices [8]. That being said, the authors do demonstrate the utility of this apparatus in the analysis of nitroaromatics in a river water sample matrix. More detailed reviews related to the analysis of nitroaromatics using both

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capillary and microchip electrophoresis in non-seawater matrices are available in the literature [13,14].

Several groups have demonstrated that direct injection of seawater in capillary electrophoresis is not always problematic provided that the analytes of interest are charged species [15–20]. The sensitive detection of anions, including iodide and phosphate, directly from seawater is possible via transient isotachophoresis wherein the chloride in the seawater matrix serves as either the leading or trailing electrolyte [15–20]. Alas, this preconcentration mode is unavailable for the nitroaromatic explosives and their degradation products, as they are neutral compounds at the pH of seawater. In order to address the issue of sensitivity of neutral analytes in very high conductivity matrices, it is necessary to develop appropriate injection/separation schemes robust enough to tolerate the presence of the seawater matrix. This work presents practical considerations when sampling directly from seawater matrices. The use of high surfactant concentrations and long electrokinetic injections allows for an order of magnitude improvement in detection limits for individual explosives in seawater over previous methods, and, additionally, permits the direct injection of seawater onto the capillary without any form of dilution.

## 2. Materials and methods

### 2.1. Reagents and standards

Sodium tetraborate, sodium cholate, sodium hydroxide, ethanol and sterile filtered seawater were purchased from Sigma–Aldrich (St. Louis, MO). Individual explosives standards including HMX, RDX, 1,3,5-trinitrobenzene (1,3,5-TNB), 1,3-dinitrobenzene (1,3-DNB), Tetryl, nitrobenzene (NB), 2,4,6-trinitrotoluene (2,4,6-TNT), 4-amino-dinitrotoluene (4-Am-DNT), 2-Am-DNT, 2,4-DNT, 2,6-DNT, 2-nitrotoluene (2-NT), 3-NT, and 4-NT were purchased from Supelco (Bellefonte, PA) at a concentration of 1000 μg/mL in acetonitrile.

Fused silica capillary was purchased from Polymicro (Phoenix, AZ) with internal diameters of 50 μm. Capillaries had an outer diameter of 360 μm and were coated with polyimide to impart mechanical stability.

### 2.2. Equipment

All separations were performed on a Beckman Coulter PACE MDQ capillary electrophoresis instrument equipped with a UV absorbance detector (Fullerton, CA). Detection occurred at 254 nm. Capillary temperature was maintained at 25 °C and the instrument was utilized at all times as per manufacturer recommendations.

### 2.3. Sample preparation

Explosive samples were prepared by diluting the purchased standards (1000 μg/mL) in 1.6 mL of the appropriate sample matrix. Final sample concentration ranged from 10 mg/L to 100 μg/L. Samples were prepared at the beginning of each day from the stock solutions.

### 2.4. Background electrolyte preparation

Separation BGEs for MEKC were prepared from stock solutions of sodium tetraborate (100 mM) and cholate (500 mM). The sodium cholate-containing BGE (20 mL) was prepared by mixing the appropriate amount of tetraborate and sodium cholate stock solutions to give a final concentration of 10 mM tetraborate and 80–240 mM cholate; 10% (v/v) ethanol was included. The addition of ethanol improved the resolution of several positional isomers, specifically the two DNTs and the three NTs. The pH was not adjusted, and the final pH of the 20 mL solution was typically 9.1. Table 1 shows the conductivity (mS/cm) of the BGE's used in this work along with the conductivity of seawater and some seawater dilutions.

**Table 1**
Conductivity of BGEs and sample matrices used in this work. Conductivity is determined by measuring current of solution in capillary at an applied voltage of 1 kV.

| Background electrolytes | Conductivity (mS/cm) |
|---|---|
| 10 mM sodium tetraborate, 80 mM sodium cholate, 10% (v/v) ethanol | 4.1 |
| 10 mM sodium tetraborate, 120 mM sodium cholate, 10% (v/v) ethanol | 5.4 |
| 10 mM sodium tetraborate, 160 mM sodium cholate, 10% (v/v) ethanol | 6.5 |
| 10 mM sodium tetraborate, 200 mM sodium cholate, 10% (v/v) ethanol | 7.6 |
| Seawater | 54.8 |
| 50% seawater | 29.9 |
| 25% seawater | 16.0 |
| 10% seawater | 7.0 |
| 5% seawater | 3.7 |

### 2.5. Injection and separation

All separations were performed on a 31.2 cm, 50 μm I.D. capillary (21.2 cm effective length). This is the shortest capillary length allowable in the MDQ instrument and was chosen to keep separation times short. Prior to the separation, the column was conditioned with 1 M NaOH for 1 min at 30 psi, followed by 1 min with MilliQ water (Billerica, MA) at 30 psi. Finally, the column was flushed with the cholate-containing BGE for 2.5 min at 30 psi. Sample was either injected hydrodynamically at 1 psi for some time to produce a plug of a desired length or, alternatively, injected for some time electrokinetically at a constant voltage of 10 kV. A small plug of BGE was injected after the sample injection (0.5 psi for 5 s) to prevent electrodispersion of the sample into the BGE vial at the inlet side of the capillary. Upon completion of the injections, the vial at the inlet side of the column was replaced with sodium cholate-containing BGE; a separation voltage of 10 kV was then applied.

## 3. Results and discussion

### 3.1. Hydrodynamic versus electrokinetic injection

In order to understand how one might improve direct sampling from seawater, it is first necessary to understand how direct sampling affects existing separations. We have previously used a BGE of 10 mM sodium tetraborate (10 mM with respect to tetraborate), 80 mM sodium cholate, and 10% (v/v) ethanol for the separation of nitroaromatics [2]. This BGE allows for the separation of 12 of 14 nitroaromatic explosives and their degradation products outlined in EPA method 8330. Fig. 1A shows the separation of 10 ppm of three nitroaromatics (TNT, 2,4-DNT, and 4-Amino-2,6-DNT) in seawater, as a function of increasing plug length, injected hydrodynamically. The shortest plug length, 10 mm, allows for the separation of the three explosives mixture, although fronting of the analyte peaks is noted. With increasing plug length, the least retained peak, TNT, is lost to the system peak associated with the seawater matrix, the middle peak, 2,4-DNT, broadens significantly, and the fronting observed in the shortest plug length experiment becomes even more obvious for the most retained peak, 4-Amino-2,6-DNT. It is likely that the significant discontinuity between the sample matrix and the BGE is disrupting the interface between the sample zone and the BGE. We have observed similar peak shapes in our previous efforts to understand the role of micelle stacking on analyte preconcentration in MEKC when the

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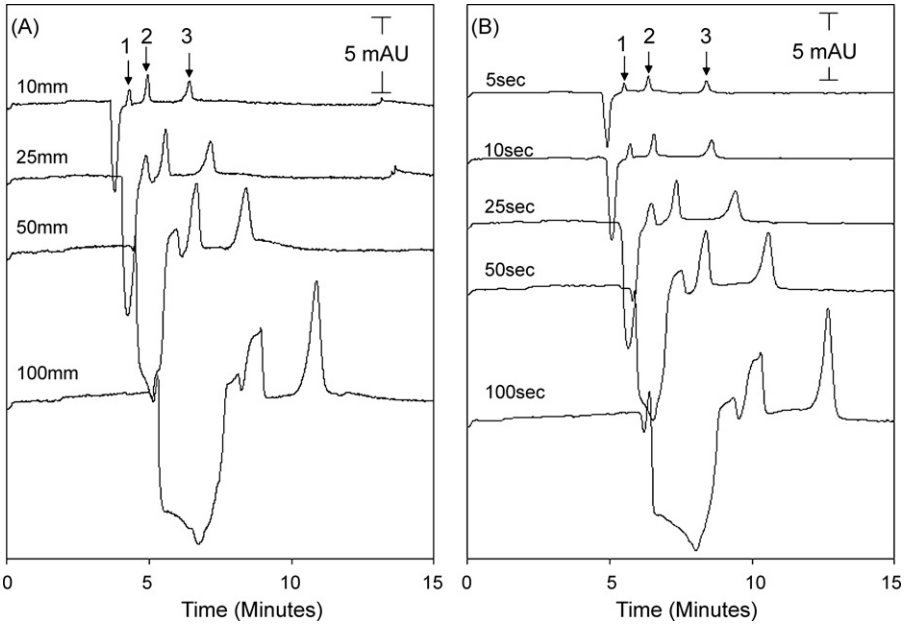


**Fig. 1.** (A) Hydrodynamic injection of three nitroaromatics (indicated by arrows) at 10 ppm. Sample plug length (mm) is indicated with the corresponding trace. (B) Electrokinetic injection of three nitroaromatics. Injection time (at an applied voltage of 10 kV) is indicated with the corresponding trace. Elution order is (1) 2,4,6-TNT, (2) 2,4-DNT, (3) 4-Amino-2,6-DNT. Sample matrix is seawater. BGE is 10 mM sodium tetraborate, 80 mM sodium cholate, 10% (v/v) ethanol. Separation voltage is 10 kV (normal polarity).

sample matrix was maintained at a higher conductivity than the BGE [8].

In order to mitigate this potential disruption, electrokinetic injection was alternatively evaluated as a method for introducing sample into the capillary. Electrokinetic injection has many potential benefits, including an inherent bias under normal polarity against the introduction of anions, i.e. $Cl^-$; it is possible that by limiting the injection of potentially disruptive anions, we can mitigate the observed fronting problem. In addition, Palmer et al. demonstrated that the use of an electrokinetic injection in MEKC can allow for the equivalent of multiple column volumes of sample [21].

Unfortunately, the use of an electrokinetic injection does not allow for any significant improvements with respect to the observed peak fronting. Fig. 1B shows the corresponding separation of the three analytes electrokinetically injected at 10 kV from 5 to 100 s. There was no appreciable change in peak asymmetry. It is useful to compare the longest electrokinetic injection (100 s at 10 kV) to the hydrodynamic injection (100 mm), as these electropherograms are representative of large sample injections wherein the total amount of analyte injected, as indicated by the peak area, is within 10% of each other (the hydrodynamic injection peak area is greater than the electrokinetic injection). Two interesting features of note were observed. First, the peak heights using the long electrokinetic injection increased approximately 7% over the corresponding long hydrodynamic injections. Secondly, while the 100-s electrokinetic injection at 10 kV allowed for a nearly equivalent separation to the 100 mm hydrodynamic injection, the peak separation was much improved. The separation between 2,4-DNT and 4-Amino-2,6-DNT was 1.93 min for the hydrodynamic injection, while the electrokinetic injection had a separation span of 2.39 min. This is certainly due to differences associated with the effective length of the capillary when comparing the hydrodynamic injection to the electrokinetic injection. The boundary between the sample zone and the BGE is only 10 cm from the detection point in the 100 mm hydrodynamic injection (accounting for the length of the sample plug), while for the electrokinetic injection, that distance is somewhat longer as the boundary is formed directly at the capillary inlet when injection begins and moves at the velocity of the cholate micelle through the column. Based upon analytes average migration times, the effective length of the column for the 100 s/10 kV injection is 14 cm. The use of long electrokinetic injections appears to be more appropriate for the analysis of explosives in seawater, allowing for both improved analyte preconcentration and resolution.

### 3.2. Effect of increasing surfactant concentration

Issues with respect to resolving power, as it relates to peak shape, have been addressed by several groups. Of particular note, Palmer et al. observed peaks flattening out when the efficacy of the stacking mechanism plateaus [9,21], and Davis and co-workers have extensively studied peak asymmetry issues and attributed fronting to neutral solute overload [22–25]. The separation performance resulting from increasing surfactant concentration in the BGE is shown in Fig. 2 for a 100-s electrokinetic injection at 10 kV. Clearly, the fronting subsides as the sodium cholate concentration increases. The peak asymmetry transitions from an average value of 0.76 to 0.95, when comparing the 80 mM cholate BGE to the 200 mM cholate BGE ($n = 5$). An increase in surfactant concentration in the BGE clearly helps mitigate the problems associated with both peak broadening and fronting by shifting the equilibrium between analyte and micelle towards the analyte/micelle complex. Interestingly, the injection efficiency, or mass of analyte injected in a defined amount of time (as measured by peak area), increases with increasing cholate concentration, as well.

The increase in peak area is a notable result since electroosmotic flow carries analyte into the capillary and the linear velocity of EOF decreases with increasing surfactant concentration. Those velocities are 4.81, 4.34, 3.91, and 3.59 cm/min for 80, 120, 160, and 200 mM cholate-containing BGE, respectively. It was necessary to ensure that the changes in injection efficiency were not due to the use of electrokinetic injections. Fig. 3A shows the peak area of 2,4-DNT (5 ppm) as a function of increasing cholate concentration in the BGE for a 20 mm sample plug (~38 nL) injected hydrodynamically. By injecting hydrodynamically, any bias due to differences in EOF velocity are eliminated. Clearly, as the cholate concentration in the BGE increases, the peak area of the 2,4-DNT

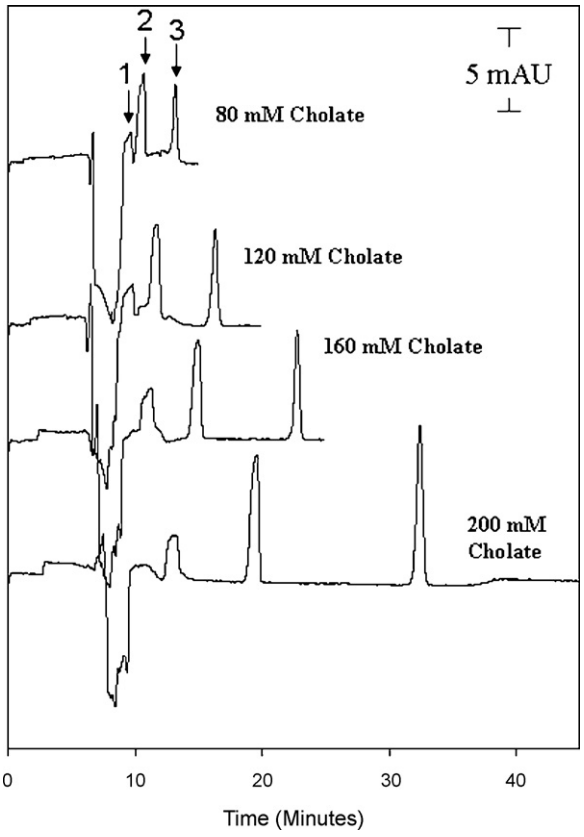


**Fig. 2.** The effect of sodium cholate concentration on separation of three nitroaromatics (indicated by arrows) at 10 ppm. Sodium cholate concentration in background electrolyte (mM) is indicated with the corresponding trace. Injection time (at an applied voltage of 10 kV) is 99 s. Elution order is (1) 2,4,6-TNT, (2) 2,4-DNT, (3) 4-Amino-2,6-DNT. Sample matrix is seawater. BGE is 10 mM sodium tetraborate, 80–200 mM sodium cholate, 10% (v/v) ethanol. Separation voltage is 10 kV (normal polarity).

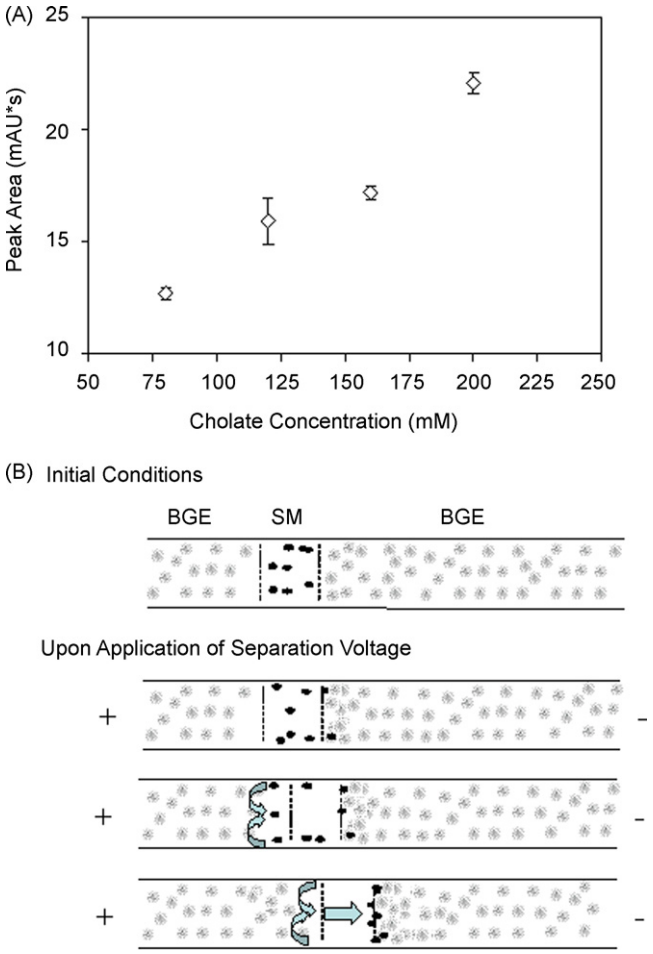


**Fig. 3.** (A) Peak area of 2,4-DNT (5 ppm) as a function of increasing cholate concentration in the background electrolyte. Sample matrix is seawater. BGE is 10 mM sodium tetraborate, 80–200 mM sodium cholate, 10% (v/v) ethanol. Separation voltage is 10 kV (normal polarity). Sample was injected hydrodynamically at 1 psi. The sample plug length is 20 mm. (B) Proposed mechanism for nitroaromatic desorption from the capillary wall.

increases. In fact, over 70% more analyte is present when comparing the use of 200–80 mM sodium cholate. Given the high conductivity of the sample matrix, it is possible that electrodispersion of the sample zone out of the column occurs during the early moments of separation. This dispersion would be exacerbated in the lower concentration cholate-containing BGEs, as the conductivity difference between the sample matrix and BGE is larger. As noted in Section 2, a small plug of BGE is always placed behind the sample plug, so that the sample is not at the very end of the capillary at the start of the separation. The 0.5 psi, 5-s injection of BGE results in a 2 mm long plug. In order to verify that electrodispersion out of the inlet side of the capillary was not occurring, this BGE length was increased up to 5 cm. The same trend of increasing peak area as a function of cholate concentration in the BGE was maintained for 2,4-DNT regardless of sample plug length (5–100 mm) or the length of BGE injected after the sample plug (2–500 mm)—data not shown.

Having eliminated electrokinetic injection bias and electrodispersion as potential explanations for the increasing sample peak area with increasing BGE cholate concentration, we next considered analyte adsorption onto the capillary wall. In MEKC separation systems where high EOF is present, analytes in the sample zone will interact with the anionic micelles already in the capillary on the detector-side of the sample plug. In the absence of any adsorption on the capillary wall, all analyte should be available for preconcentration at the detector-side of the sample zone. If adsorption does occur, however, the micelle-containing BGE at the inlet side of the sample plug would cause the analyte to desorb from the capillary wall. Analyte would then migrate through the sample zone to the sample zone/BGE interface on the detector-side of the sample zone. This mechanism is depicted in Fig. 3B. If this were the case, we can surmise that the 80 mM cholate-containing BGE simply does not have the micelle concentration necessary to successfully desorb all of the analyte from the capillary surface, whereas a higher concentration of cholate would be sufficient to complete this desorption.

To test this hypothesis, two experiments were designed to demonstrate analyte interaction with the inlet side sample matrix/BGE interface. First, the capillary was filled with cholate-containing BGE (80–200 mM), but the inlet vial was maintained at 80 mM cholate. Seawater containing 2,4-DNT was injected hydrodynamically followed by a small plug of inlet side BGE. Fig. 4A shows that the peak area does not fluctuate when 80 mM cholate is maintained in the inlet vial. Conversely, Fig. 4B shows a second experiment, in which the capillary was filled exclusively with 80 mM cholate-containing BGE, while the inlet vial had its cholate concentration varied from 80 to 200 mM. In this experiment, the peak area increases as the cholate concentration in the inlet vial increases. It is concluded from these results, therefore, that the BGE in the inlet side vial plays a critical role in the amount of analyte detected, and that adsorption to the capillary wall is the likely source for the observed peak area disparity.

This adsorption of explosives on the capillary wall is either a product of the high conductivity of the sample matrix or, alternatively, a property intrinsic to this analyte set. Fig. 4C shows the peak

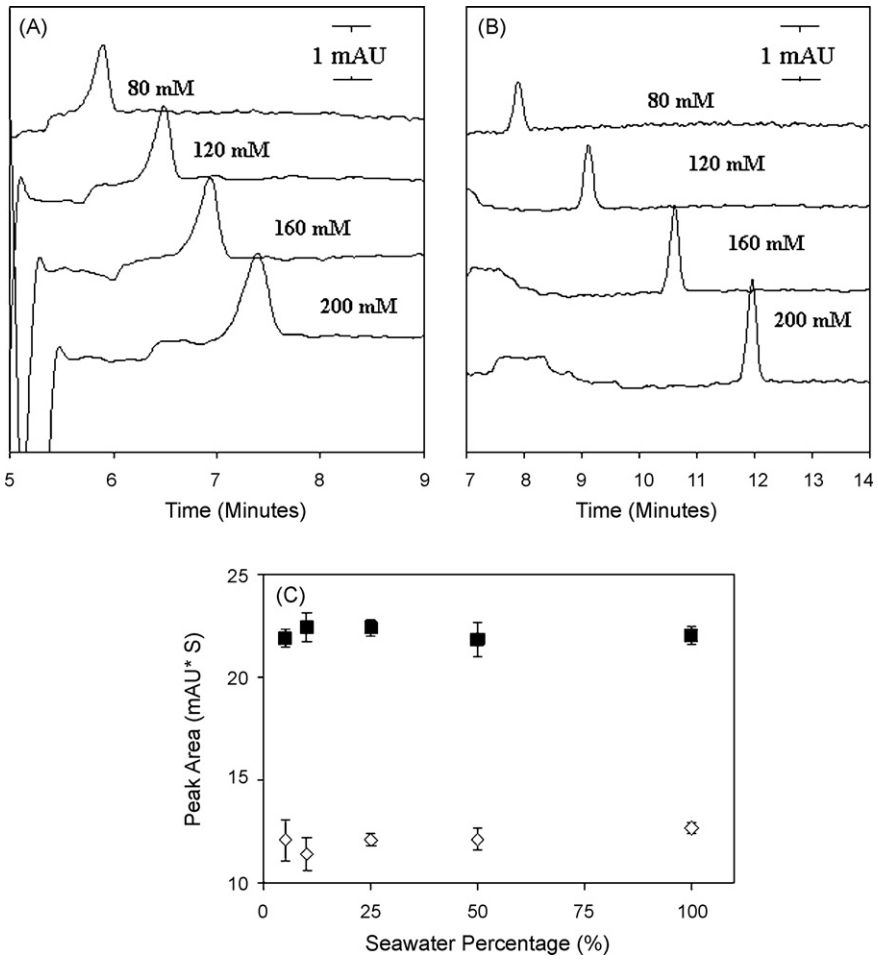


**Fig. 4.** (A) Peak associated with a 20 mm long plug of 2,4-DNT (5 ppm). Capillary is filled with (from top to bottom) 80, 120, 160, and 200 mM cholate-containing BGE. The inlet vial is filled with 80 mM cholate-containing BGE. (B) Peak associated with 20 mm long plug of 2,4-DNT (5 ppm). Capillary is filled with 80 mM cholate-containing BGE in all traces. The inlet vial is filled with (from top to bottom) 80, 120, 160, and 200 mM cholate-containing BGE. Sample matrix is seawater. (C) Peak area associated with sample prepared in 5–100% seawater for 200 and 80 mM cholate-containing BGE. BGE is 10 mM sodium tetraborate, 80–200 mM sodium cholate, 10% (v/v) ethanol. Separation voltage is 10 kV (normal polarity). Sample was injected hydrodynamically at 1 psi. The sample plug length is 20 mm.

area as a function of percent seawater for separations using 80 mM (◊) and 200 mM (■) cholate-containing BGEs. The peak areas for both BGEs are not statistically different from 5 to 100% seawater, indicating that under a wide range of sample matrix salt concentration nitroaromatics adsorb to the capillary wall, indicating that this adsorption is an intrinsic property of the analyte set.

### 3.3. Limits of detection when injecting from pure seawater

It has been noted, by our group and others, that stacking and separation efficiency in MEKC for a given analyte are related to the analytes affinity for the micelle [2,8,9]. When analytes have a high affinity for the micelle, they tend to pre-concentrate bet-

**Table 2**
Average limit of detection (S/N = 3) for nitroaromatics and their degradation products. ($n$ = 3), units are parts per billion. Numbers preceding analyte name indicates elution order.

| | 5-s inj. | 10-s inj. | 25-s inj. | 50-s inj. | 100-s inj. |
|---|---|---|---|---|---|
| (1) 1,3,5-Trinitrobenzene | 1970 | 1480 | - | - | - |
| (2) HMX | 8290 | 2330 | - | - | - |
| (3) 2,4,6-Trinitrotoluene | 3350 | 920 | - | - | - |
| (4) RDX | 5520 | 1730 | - | - | - |
| (5) 1,3-Dinitrobenzene | 1920 | 510 | - | - | - |
| (6) Tetryl | 4910 | 1040 | - | - | - |
| (7) Nitrobenzene | 1820 | 800 | 1080 | - | - |
| (8) 2,4-Dinitrotoluene | 1230 | 510 | 520 | 190 | - |
| (9) 2,6-Dinitrotoluene | 1850 | 670 | 400 | 670 | - |
| (10) 4-Nitrotoluene | 3700 | 1450 | 780 | 370 | - |
| (11) 2-Nitrotoluene | 2470 | 1410 | 460 | 290 | 170 |
| (12) 3-Nitrotoluene | | | | | |
| (13) 2-Amino-4,6-dinitrotoluene | 1670 | 1090 | 300 | 220 | 70 |
| (14) 4-Amino-2,6-dinitrotoluene | | | | | |

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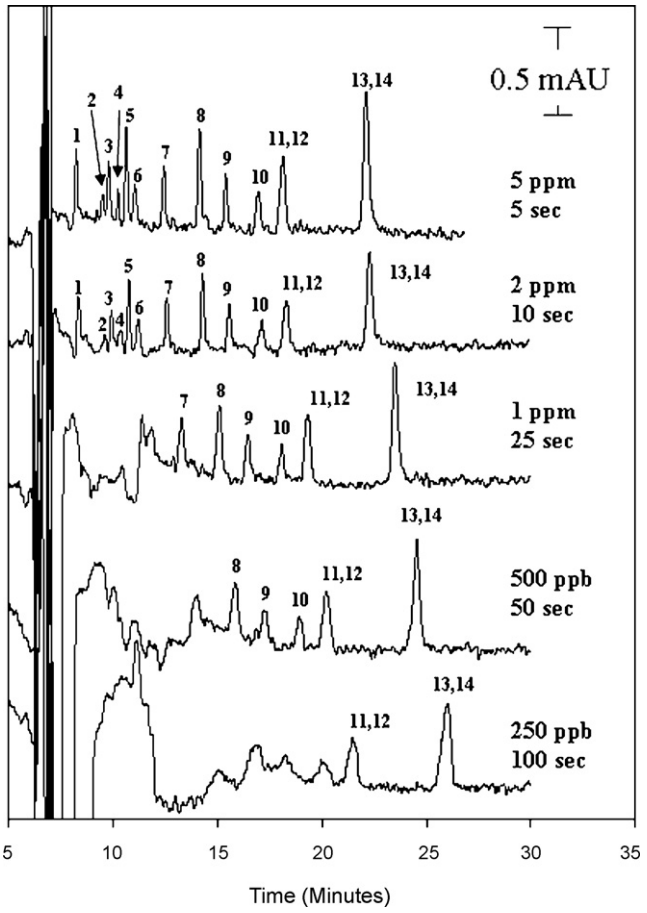


**Fig. 5.** Separation of 14 nitroaromatic explosives and their degradation products. Sample concentration and electrokinetic injection time (at an applied voltage of 10 kV) are indicated by the corresponding trace. Elution order is (1) 1,3,5-trinitrobenzene, (2) HMX, (3) 2,4,6-trinitrotoulene, (4) RDX, (5) 1,3-dinitrobenzene, (6) Tetryl, (7) nitrobenzene, (8) 2,4-dinitrotoluene, (9) 2,6-dinitrotoluene, (10) 4-nitrotoluene, (11) 2-nitrotoluene, (12) 3-nitrotoluene, (13) 2-amino-4,6-dinitrotoluene, (14) 4-amino-2,6-dinitrotoluene. Sample matrix is seawater. BGE is 10 mM sodium tetraborate, 200 mM sodium cholate, 10% (v/v) ethanol. Separation voltage is 10 kV (normal polarity).

ter and maintain a Gaussian peak shape longer, even after long injection times. Fig. 5 illustrates the effects of injection time and concentration on the ability to resolve and detect nitroaromatics in seawater. The top trace shows a 5-s injection of nitroaromatics at a concentration of 5 ppm for all analytes. With the exception of 2-NT and 3-NT co-eluting and 2-Amino-4,6-DNT and 4-Amino-2,6-DNT co-eluting, all analytes are resolved. Since the unresolved NT and amino-DNT pairs share similar molar absorptivities, respectively, the effective concentration of these two peaks is 10 ppm. LOD's (S/N = 3) range from 8.3 ppm for HMX to 1.2 ppm for 2,4-DNT. With increasing injection time, the ability to resolve the least retained peaks diminished, while the LODs for the more retained peaks improves. For a 10-s injection, all analytes remain resolvable, with HMX again having the highest LOD of 2.3 ppm, while both 1,3-DNB and 2,4-DNT have LODs near 500 ppb. As the injection time increases, the ability to resolve the least retained peaks becomes significantly hindered. That being said, detection limits significantly improve for many of the most retained peaks; the LOD of 2,6-DNT is calculated at approximately 400 ppb, and the co-eluted 2-amino-4,6-DNT and 4-amino-2,6-DNT have an LOD of 300 ppb for a 25-s injection. For 100-s injections, only the most retained analytes, the peaks associated with 2-NT/3-NT and 2-amino-4,6-DNT/4-amino-2,6-DNT peaks are detectable, with LODs of 170 and 70 ppb, respectively. Table 2 summarizes the average calculated limits of detection (S/N = 3, $n = 3$) for the nitroaromatic explosives and their degradation products as a function of the injection time. Note that Table 2 does not summarize individual, single analyte detection limits, but, instead, detection limits derived when analyzing a complex seawater sample containing 14 nitroaromatics and their degradation products. For those cases when an analytes peak was not fully resolved from its neighbor, a hyphen was inserted to indicate the failure to resolve this peak and accurately determine a detection limit.

Our previous efforts focused on using separation conditions that demonstrated a tolerance to sampling from high-salt containing matrices. In that work, we determined that sensitivity was improved by diluting the sample to 25% of its original concentration. Even though the sample concentration was reduced, the stacking mechanism was more tolerant to the lower conductivity sample matrix and the net effect was lower limits of detection. In order to compare this new method with the old, the sample was diluted to 25% seawater and analyzed using conditions described in our previous work [2]. Those conditions are a background elec-

**Table 3**
Comparison of peak heights using direct electrokinetic injection of pure seawater to pressure injection of 25% seawater. Peak heights are presented as "new method/old method". Units are μAU.

| | Injected conc. (ppb) | Height (10 s inj/10 mm inj) | Height (50 s inj/20 mm inj) | Height (100 s inj/100 mm inj) |
|---|---|---|---|---|
| Original sample concentration: 4000 ppb | | | | |
| 2,4,6-Trinitrotoluene | 4000/1000 | 780/– | 860/– | –/– |
| Nitrobenzene | 4000/1000 | 710/260 | 1390/300 | 1340/350 |
| 2,4-Dinitrotoluene | 4000/1000 | 1190/360 | 3130/530 | 3030/680 |
| 3-Nitrotoluene | 4000/1000 | 510/200 | 2040/330 | 2570/730 |
| 4-Amino-2,6-dinitrotoluene | 4000/1000 | 700/290 | 3460/500 | 4300/770 |
| Original sample concentration: 1000 ppb | | | | |
| 2,4,6-Trinitrotoluene | 1000/250 | 200/– | 140/– | –/– |
| Nitrobenzene | 1000/250 | 210/90 | 410/105 | 390/– |
| 2,4-Dinitrotoluene | 1000/250 | 320/80 | 450/130 | 820/– |
| 3-Nitrotoluene | 1000/250 | 170/– | 530/– | 710/– |
| 4-Amino-2,6-dinitrotoluene | 1000/250 | 230/70 | 740/110 | 1230/– |
| Original sample concentration: 500 ppb | | | | |
| 2,4,6-Trinitrotoluene | 500/125 | 110/– | 160/– | –/– |
| Nitrobenzene | 500/125 | 110/– | 210/– | 360/– |
| 2,4-Dinitrotoluene | 500/125 | 150/– | 390/– | 430/– |
| 3-Nitrotoluene | 500/125 | 100/– | 260/– | 370/– |
| 4-Amino-2,6-dinitrotoluene | 500/125 | 130/– | 380/– | 670/– |

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trolyte of 10 mM borate, 80 mM cholate, pH 9.1; 75 μm I.D., 360 μm O.D., 60.2 cm total length (50 cm effective length) capillary; sample injected for a specified sample plug length at 0.5 psi followed by a 3-s, 0.5 psi injection of background electrolyte; applied voltage is 30 kV, which resulted in an operational current of approximately 120 μA. Table 3 presents a comparison of peak heights obtained for the new method compared to the old method (the baseline noise for both method were equivalent so a direct comparison of peak height is possible even though the capillary diameters and BGE compositions are different).

For a 4000 ppb sample in seawater, all five components are detectable at the shortest injection time using the newly optimized separation conditions. Sample dilution to 25% seawater and analysis via our previously described method allows for the detection of 4 of 5 analytes. TNT is not detected at any injection length due to poor stacking efficiency for the least retained analytes and proximity to system peaks. When the sample concentration is reduced to 1000 ppb, the newly optimized conditions continue to outperform our previous efforts. In this case, all 5 analytes are detected for 10- and 50-s injections, with the ability to detect TNT lost with a 100-s injection. Only the 10 and 20 mm injection plug lengths are effective using the old separation conditions for 3 of 5 of the analytes of interest. Finally, when the sample is reduced to a concentration of 500 ppb, the old method does not allow for the detection of a single component regardless of injection plug length.

## 4. Conclusions

The ability to directly inject long sample plugs from seawater matrices for the analysis of nitroaromatic explosives was demonstrated. The use of electrokinetic injection coupled with BGE optimization (200 mM sodium cholate) allowed for detection limits as low as 70 ppb for a mixture of 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene. Improved detection limits are attributed to a combination of limiting analyte fronting during the separation by increasing the micelle concentration in the BGE and desorption of analyte from the capillary wall due to that same high micelle concentration. The adsorption of nitroaromatics does not appear to depend on the seawater, sample matrix concentration and requires one to consider that neutral analytes will interact with both the detection and the inlet side sample matrix/background electrolyte interfaces.

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